



UNIVERSITI PUTRA MALAYSIA

***EFFECTS OF S-ADENOSYL-L-HOMOCYSTEINE AND TRICHOSTATIN A
ON DEVELOPMENTAL COMPETENCE, EPIGENETIC MODIFICATION,
AND GENE EXPRESSION IN CLONED CATTLE EMBRYOS***

SHAHRAM JAFARI

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By

SHAHRAM JAFARI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of
Philosophy**

August 2012

THIS THESIS IS DEDICATED TO
MY WIFE, MY MOTHER, MY DAUGHTER
WITH
LOVE AND GRATITUDE
AND ALSO TO
THE CHILDREN WHO HAVE THE ABILITY
BUT
NOT THE FACILITY AND OPPORTUNITY IN EDUCATION

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairman: Assoc. Professor Halimatun bt Yaakub, PhD

Faculty: Agriculture

With regards to low efficiency of the somatic cell nuclear transfer (SCNT) procedure the hypothesis that epigenetic marks of somatic donor cells are responsible for this inefficiency is proposed. Epigenetic marks such as DNA methylation and histone acetylation are two major participants in nuclear reprogramming which can be used as indicators to assess SCNT efficiency. For this reason treating somatic cells or embryos with epigenetic drugs immediately after fusion or activation may improve the developmental rate of reconstructed embryos. Therefore, in this study we investigated the effect of S-adenosyl-L-homocysteine (SAH), a reversible inhibitor of DNA methyltransferases (DNMTs), at different intervals post SCNT on developmental competence, epigenetic status and gene expression of bovine cloned embryos. Treatment with 1mM SAH for 12 hours resulted in 54.6% blastocyst production which was significantly

higher ($P<0.05$) than *in vitro* fertilized (IVF) embryos (37.2%), cloned embryos treated with SAH for 72 hours (31.0%) and control cloned embryos (34.6%).

Intensity of DNA methylation in cloned embryos treated with SAH for 48 h resembled IVF and was significantly lower ($P<0.05$) than other SCNT groups. The histone H3 Lysine 9 (H3K9) acetylation levels of all SCNT groups were significantly lower ($P<0.05$) than the IVF group. The fluorescent intensity of EGFP-POU5F1 reporter gene at all intervals of SAH treatments, except for the 72 hours, was significantly higher ($P<0.05$) than non-treated SCNT embryos. Real-time analysis of gene expression in cloned blastocysts revealed significantly higher ($P<0.05$) expression of POU5F1 compared with IVF. There was no effect ($P>0.05$) of either embryo production method (SCNT vs. IVF), or among SAH treatment interval on the expression of BCL2 gene. On the contrary, treatment with SAH resulted in significant increase ($P<0.05$) in VEGF expression in comparison with IVF and control SCNT, except for cloned embryos treated with SAH for 24 hours. It was suggested that time interval of DNA methyltransferases (DNMTs) inhibition may have important consequences on the different features of bovine cloned embryos and the improving effects of DNMTs inhibition on developmental competency of cloned embryos is restricted to a specific period of time preceding *de novo* methylation stage. The effect of 0.05 μ M Trichostatin A (TSA), a histone deacetylase inhibitor, post-fusion for 12 h on the diverse aspects of developmental competency in SCNT embryos was investigated. The results of this study showed TSA treatment of SCNT embryos for 12 h after activation, significantly ($P<0.05$) improve blastocysts rate.

A significant reduction and increase ($P < 0.05$) were observed in analysis of DNA methylation and histone acetylation levels respectively in the blastocyst stage of the TSA-treated group which is similar to IVF-derived blastocysts. Finally, examination of blastocysts on day 7 revealed the highest expression of OCT4 in the TSA-treated group ($P > 0.05$), while there were no significant changes observed in expressions of BCL2 and VEGF.

The results of this study indicated that post-fusion treatment with SAH has a time dependent effect on DNA-methylation and histone-acetylation, developmental competence and gene expression of the cloned embryos. The results of this study also showed that treatment of SCNT embryos with TSA after activation increased the capability of the embryos to develop to the blastocyst stage and TSA-treated cloned blastocysts are hypomethylated and hyperacetylated and higher relative expression of POU5F1 and VEGF genes. In addition, these results might improve quality of cloned bovine embryos to produce transgenic cattle.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**KESAN S-ADENOSIL-L-HOMOSISTEINA DAN TRIKOSTATIN A TERHADAP
KECEKAPAN PERKEMBANGAN, MODIFIKASI EPIGENETIK DAN
PENGEKSPRESAN GEN DARIPADA EMBRIO BOVIN TERKLON**

Oleh

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Pengerusi : Profesor Madya Halimatun Yaakub, PhD

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Berdasarkan pada kerendahan kecekapan prosedur pemindahan nukleus sel soma (SCNT), hipotesis yang menyatakan kebanyakan penanda epigenetik sel soma penderma bertanggungjawab terhadap tidak kecekapan ini dicadangkan. Penanda epigenetik seperti pemetilan DNA dan pengasetilan histon adalah dua komponen penting dalam program semula nukleus yang boleh digunakan sebagai penunjuk untuk menilai kecekapan SCNT. Untuk tujuan ini, merawat sel-sel soma atau embrio dengan bahan kimia epigenetik sejurus selepas penggabungan atau pengaktifan mungkin boleh meningkatkan kadar perkembangan embrio yang dibentuk semula.

Oleh itu, dalam kajian ini kami mengkaji kesan SAH, suatu perencat berbalik metiltransferase DNA (DNMTs), pada selang berbeza selepas SCNT terhadap kompetensi perkembangan, status epigenetik dan pengekspresan gen daripada

embrio bovin terklon. Rawatan dengan menggunakan 1 μ l SAH selama 12 jam menghasilkan blastosista sebanyak 54.6% dimana ia lebih tinggi ($P < 0.05$) berbanding embrio yang disenyawakan secara *in vitro* (IVF: 37.2%), embrio terklon yang dirawat dengan SAH selama 72 jam (31.0%) dan embrio terklon kawalan (34.6%).

Intensiti pemetilan DNA dalam embrio terklon yang dirawat dengan SAH selama 48 jam menyerupai IVF dan lebih rendah ($P < 0.05$) berbanding kumpulan SCNT yang lain. Tahap pengasetilan H3K9 (H3K9-Ac) dari semua kumpulan SCNT nyata sekali lebih rendah ($P < 0.05$) daripada kumpulan IVF. Intensiti *fluorescent* (berpendarfluor) gen pelapor EGFP-POU5F1 pada semua selang rawatan SAH, kecuali 72 jam, nyata sekali lebih tinggi ($P < 0.05$) berbanding kumpulan kawalan. Analisis masa nyata dan pengekspresan gen dalam blastosista yang diklon menunjukkan ekspresi POU5F1 adalah lebih tinggi ($P < 0.05$) berbanding IVF. Tidak terdapat kesan ($P > 0.05$) terhadap kaedah pengeluaran embrio (SCNT vs IVF) atau selang masa rawatan SAH ke atas ekspresi gen BCL-2.

Tambahan pula, rawatan menggunakan SAH tidak menunjukkan peningkatan signifikan ($P > 0.05$) dalam pengekspresan VEGF berbanding dengan IVF dan SCNT kawalan, kecuali untuk embrio terklon yang dirawat dengan SAH selama 24 jam. Disarankan bahawa selang masa perencatan DNMTs mungkin mempunyai akibat penting terhadap ciri embrio bovin terklon. Kesan peningkatan perencatan DNMTs terhadap kompetensi perkembangan embrio terklon adalah terhad pada jangka masa tertentu sebelum tahap pemetilan *de*

novo. Dengan keputusan ini, penelitian terhadap kesan 0.05 μ M TSA, perencat diafetilasi histon, selepas gabungan kuplet selama 12 jam dan pelbagai aspek kompetensi perkembangan pada embrio yang dihasilkan secara SCNT telah dilakukan. Tiada perubahan ($P>0.05$) didapati pada peningkatan belahan, dan kadar blastosista di antara kawalan dan kumpulan yang di rawat.

Walau bagaimanapun, kadar blastosista yang lebih tinggi ($P<0.05$) telah ditunjukkan dalam kumpulan yang dirawat dengan TSA. Kedua-dua penurunan dan peningkatan yang ketara ($P<0.05$) telah di amati dalam analisis pemetilan DNA dan tahap asetilasi histon dalam peringkat blastosista dalam kumpulan yang dirawat adalah mirip dengan blastosista daripada IVF. Akhir sekali, pemeriksaan blastosista pada hari ke 7 mendedahkan pengekspresan OCT4 tertinggi pada kumpulan yang dirawat dengan TSA ($P<0.05$), manakala tiada perubahan ($P>0.05$) didapati dalam pengekspresan BCL2 dan VEGF.

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I certify that a Thesis Examination Committee has met on Date of Viva Voce to conduct the final examination of Shahram Jafari on his thesis entitled. "Effects of S-adenosylhomocysteine and Trichostatin A on Developmental Competence, Epigenetic Modification and Gene Expression of Cloned Cattle Embryos" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the degree of Doctor of Philosophy.

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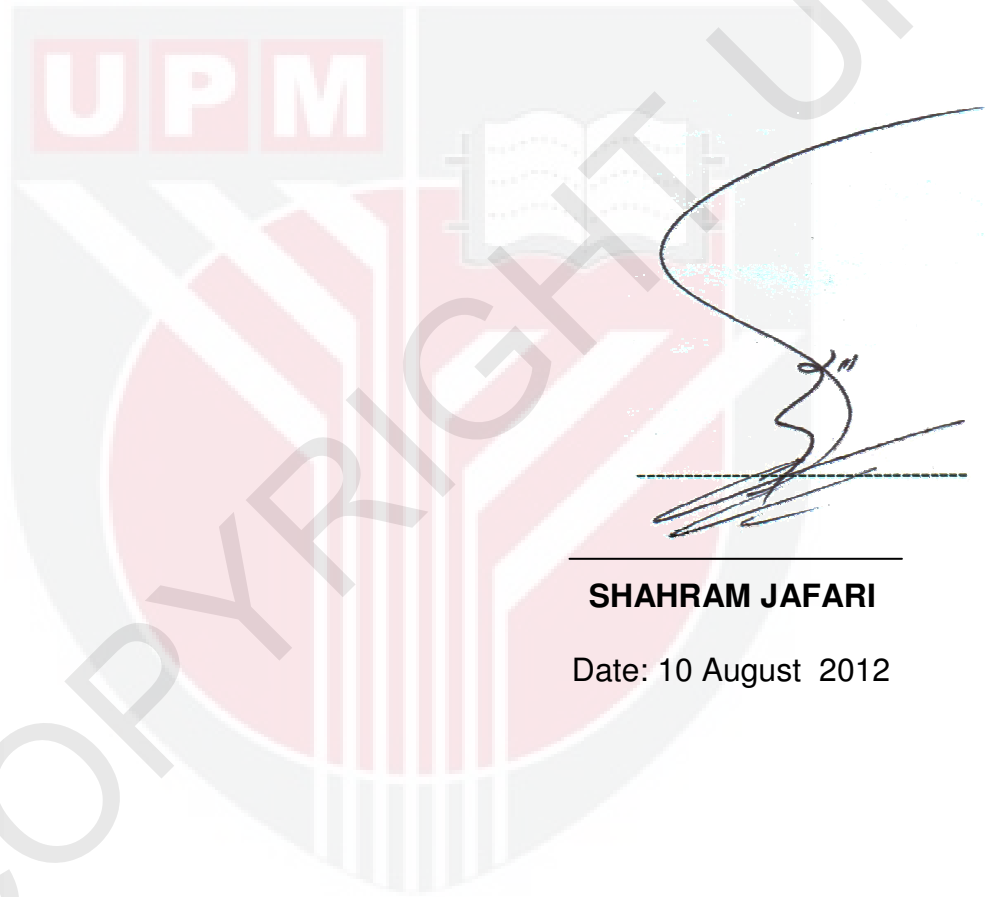
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DECLARATION

I declare that the thesis is my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously and it is not concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.



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Date: 10 August 2012

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3. **EFFECTS OF S-ADENOSYL-L-HOMOCYSTEINE AND TRICHOSTATIN A ON DEVELOPMENTAL COMPETENCY, EPIGENETIC MODIFICATION AND GENE EXPRESSION IN CLONED CATTLE EMBRYOS**

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